Design, Synthesis and Preliminary Antimicrobial Evaluation of New Derivatives of Cephalexin

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Abstract

There is a continuous and massive need for newer cephalosporins that should have resistance against β-lactamases and can be used orally. An approach of using cephalexin, as a well-studied and potent antibacterial compound is considered to prepare new designed derivatives. These derivatives include the incorporation of amino acid moiety linked through an amide bond with the α-amino group of cephalexin. Certain aliphatic amino acids were used, such as glycine, alanine, valine and proline. The chemical structures of these derivatives were confirmed by IR spectroscopy and elemental analyses. All the synthesized compounds were subjected for preliminary evaluation of antimicrobial activity using well diffusion method, against certain microbes. Most of the synthesized compounds were found to possess significant antibacterial activities. Compound 1 (125 µg and 250µg) showed significant activity against *P. aeruginosa*. Compound 2 (125 and 250µg) exhibited significant activity against *P. aeruginosa* and *Bacillus cereus*. Compound 3 (125 and 250µg) demonstrated very significant activity against *E. coli*, *P. aeruginosa* and *Bacillus cereus* and slight activity towards *S. aureus.* Compound 4 (250µg) showed significant activity against *P. aeruginosa* and no antibacterial activities against *E. coli*, *S. aureus* and *Bacillus cereus*, as compared with cephalexin as the standard compound.

Keywords: Cephalosporins, Cephalexin, Glycine, Alanine, Valine, Proline.

تصميم وتحضير والتقييم الاولي للفعالية المضادة للبكتيريا لمشتقات جديدة لعقار السيفالكسين

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الخلاصة

اتضح من الدراسات والبحوث العلميه بان هنالك حاجه كبيرة لمضادات حيويه من نوع السيفالوسبورينات المقاومه للبكتريا المنتجة لانزيمات البيتالاكتميز والتي قد تستعمل عن طريق الفم. استخدم السيفالاكسين لتحضير مشتقات جديدة بواسطة ربط الاحماض الامينيه عن طريق مجموعه الكاربوكسيل بمجموعة الامين الاوليه (حره) الموجوده في جزيئة السيفالكسين وتكوين اصرة امايد باستعمال الاحماض الامينية الاليفاتية مثل الكلايسين ،الألنين ، فالين والبرولين. تم تشخيص التراكيب الكيمياوية لهذه المشتقات بواسطة التحليل الطيفي (الاشعة تحت الحمراء) والتحليل الدقيق للعناصر (الكاربون والهيددروجين والنتروجين) وكانت النتائج مطابقه للتركيبات الكيمياويه المفترضه. تمت دراسة الفعاليه المضادة للبكتريا باستعمال بعض الميكروبات المرضية واظهرت النتائج بان المركب ١ (١٢٥-٢٥٠ مايكروغرام) لديه فعاليه متميزة ضد الزائفة الزنجارية ، بينما المركب ٢ (١٢٥-٢٥٠ مايكروغرام) فقد اظهر فعاليه ضد الزائفة الزنجارية والبكتريا العصوية الشمعية. في حين ان المركب ٣ (١٢٥-٢٥٠ مايكروغرام) اظهر فعاليه جدا متميزة ضد الزائفة الزنجارية والبكتريا العصوية الشمعية و الاشريكية القولونية. اما المركب ٤ (٢٥٠ مايكرغرام) فقد اظهر فعاليه متميزة ضد الزائفة الزنجارية ولم يظهر أي فعاليه لبقيه المايكروبات وقد استعمل عقار السيفالكسين كمادة قياسية للمقارنه.

الكلمات المفتاحية:السيفالوسبورين ،السيفالكسين ، كلايسين،الالنين ، فالين والبرولين.

Introduction

The wide use of antibiotics in man and animals and their extensive use in areas other than the treatment and prophylaxis of diseases have resulted in a serious problem of drug resistance. More and more bacterial strains have become resistant to available drugs. A relation between the structure of the complexes and their anti-bacterial activity can be observed [(1)].

In the last two decades, antimicrobial resistance has become one of the greatest health problems, mainly in hospitals [(2)].

Despite the continuous development and introduction of new antibiotics, resistance continues to increase progressively in several microbes [(3)]. Preparation of different semisynthetic derivatives of cephalosporins based on structure-activity relationship has been one of the best approaches. Intensive search for new cephalosporins that may have broader antibacterial spectrum and resistance toward β-lactamase–producing bacteria and could be used orally are of great interest [(4)]. Cephalexin is a β- lactam antibacterial and has bactericidal action and acts similarly to

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benzyl penicillin by inhibiting synthesis of the bacterial cell wall. It is most active against G (+) cocci and has moderate activity against some G (-) bacilli. Sensitive G (+) cocci includes both penicillinase- and non-penicillinase-producing *staphylococci*, although methicillin-resistant *staphylococci* are resistant; most *streptococci* are also sensitive, but not penicillin-resistant *Streptococci Pneumonia*; *Enterococci*, which are usually resistant. Some G (+) anaerobes are also susceptible. Cephalexin is usually inactive against *Listeria monocytogenes*. Among G (-) bacteria, cephalexin has activity against some *Enterobacteriaceae* including strains of *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *Salmonella* and *Shigella spp*., but not active against *Enterobacter*, *indole-positive Proteus* and *Serratia spp*. It is also active against *Moraxella catarrhalis* (*Branhamella catarrhalis*) and *Neisseria* spp., though *Haemophilus influenzae* is moderately resistant. *Bacteroides fragilis* and *P. aeruginosa* are not sensitive and neither, *mycobacteria*, *mycoplasma* nor fungi [5]. The incorporation of privileged chemical moieties, such as, amino acids has been found to have great potential in the field of antimicrobial agents. Amino acid linked to cephalexin through amide bond can be of great benefits and may add appreciable activity, resistance to β-lactamases and/ or improved pharmacokinetic properties . The proposed compounds may serve for injectable purposes, when prepared as sodium salt or could be used orally due to expected stability in aqueous acidic condition due to the presence of primary amine group at the α-carbon of the acyl side chain.

Experimental Work

Materials and Methods

General

Melting points (uncorrected) were determined using electrical melting point apparatus, Electro-thermal 9300, USA. The infrared spectra were performed in KBr disc by FT-IR spectrophotometer/ Shimadzu. Elemental micro-analyses (CHN) were performed by Euro-vector EA 3000A, Italy. Checking the purity of the products as well as monitoring the progress of the reaction was achieved by Thin layer chromatography using Silica gel F_{254} aluminum sheets, Merck, Germany.

Chemicals and Reagents

Ethyl chloroformate (ECF) was purchased from Sigma Aldrich/ Germany, Boc- glycine; Boc-L-alanine, Boc-L-valine and Boc-L-proline were purchased from Shanghai World Yang Chemical/China. Trifluoroacetic acid (TFA) was obtained from Sigma Aldrich/Germany. Cephalexin monohydrate was from SDI Samarra/Iraq

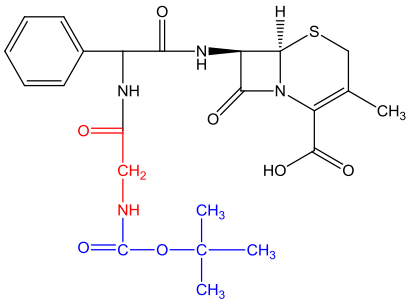
General procedure for synthesis of the intermediates of cephalexin (1a-d).

The new intermediates of cephalexin were synthesized by the mixed anhydride method [7-9], as shown in (schemes 1and 2).

Boc-amino acid (11.41 mmol) was dissolved in Tetrahydrofuran, THF (20mL) containing TEA (11.41 mmol) and this mixture was cooled in an ice bath at (-10°C). Ethyl chloroformate, ECF (11.41 mmol) was added drop wise over a period of 10 min and the mixture was continuously stirred for further 30 min. Cephalexin (11.41 mmol) in distilled water (10ml), containing TEA (11.41 mmol) was cooled to 0 °C and added at once to the above solution and the mixture was stirred for 4 hrs at -10 °C and for 2 hrs at room temperature. The solvent was evaporated and the resultant precipitate was washed with diluted HCl (0.1N) and filtered. The precipitate was collected and was washed with water several times with stirring, then washed with ether, recrystallized from ethanol/ toluene (1:9).

Synthesis of 1a, 7-(2-(2-((t-butoxy carbonyl)-amino)acetamido)-2-phenylacetamido)-3-methyl–8-oxo-5-thia-1-azabicyclo {4.2.0}oct-2-ene-carboxylate

This compound **1a** was synthesized, as previously described and as shown in (scheme 1).

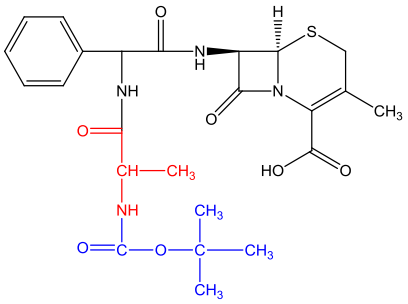


Chemical structure of compound 1a

Boc-glycine (11.416mmol, 5.75g) in THF (20mL) containing TEA (11.416 mmol, 1.153 g) was reacted with ECF (11.416 mmol, 1.24 g). Cephalexin (11.416 mmol, 4.16 g) in distilled water (10ml) containing TEA (11.416 mmol, 1.153 g) was added. The reaction mixture was treated as described earlier. The physical appearance, percent yield and R_f value are listed on table (1).

Synthesis of 1b, 7-(2-2-((t-butoxy carbonyl) amino) propanamido)-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo {4.2.0} oct-2-ene-carboxylate

This compound **1b** was synthesized, as follows and as shown in (scheme 1):

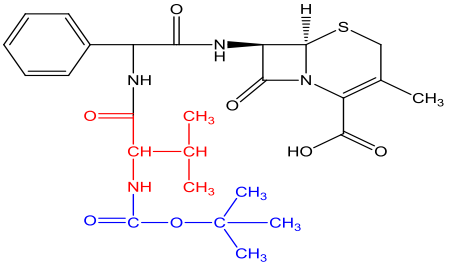


Chemical structure of compound 1b

BOC-alanine (11.416 mmol, 5.91 g) in 20 ml of THF containing TEA (11.416 mmol, 1.153g) was reacted with ECF (11.416 mmol, 1.24 g). Cephalexin (11.416mmol, 4.16 g) in distilled water (10ml) containing TEA (11.416 mmol, 1.153g) was added. The reaction mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed on table (1).

Synthesis of 1c, 7-(2-2- ((t-butoxy carbonyl) amino)-3-methylbutanamido)-2-phenyl acetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo {4.2.0} oct-2-ene carboxylate

Compound **1c** was synthesized, as follows and as shown in (scheme 1):

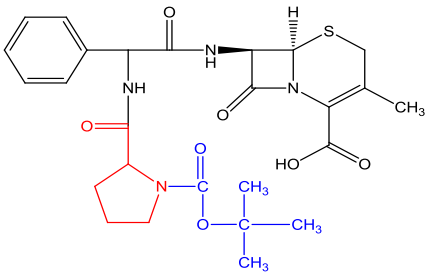


Chemical structure of compound 1c

Boc-valine (11.416 mmol, 6.23 g) in 20 ml of THF containing TEA (11.416 mmol, 1.153g) reacted with ECF (11.416 mmol, 1.24g). Cephalexin (11.416 mmol, 4.16 g) in distilled water (10ml) containing TEA (11.416 mmol, 1.153g) was added. The mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed on table (1).

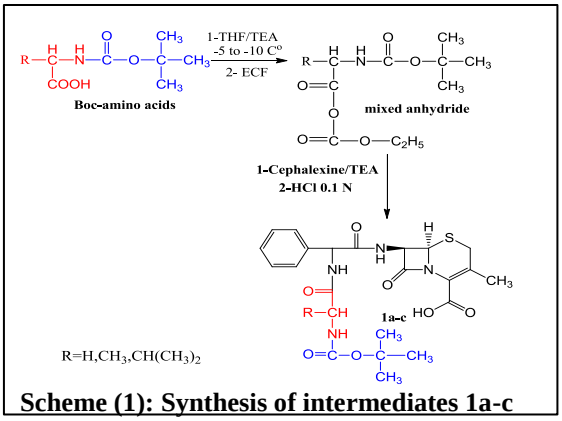
Synthesis of 1d, 7-(2-(1-(t-butoxy carbonyl) pyrrolidin-2-carboxamido)-2-phenylacet amido)-3-methyl-8-oxo-5-thia-1-azabicyclo {4.2.0} oct-2-ene-carboxylate.

Compound **1d** was synthesized, as follows and as shown in (scheme 2):

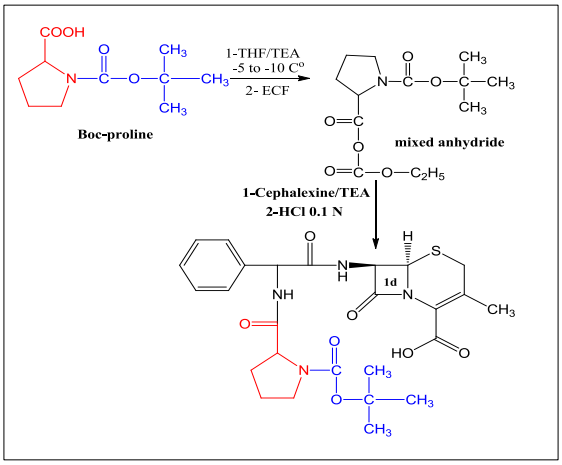


Chemical structure of compound 1d

Boc-proline (11.416mmol, 6.22g) in THF (20ml) containing TEA (11.416 mmol, 1.153g) was reacted with ECF (11.416mmol, 1.24g). Cephalexin (11.416 mmol, 4.16 g) in distilled water (10ml) containing TEA (11.416 mmol, 1.153g) was added. The mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed on table (1).



Scheme (1): Synthesis of intermediates 1a-c



Scheme (2): Synthesis of intermediate 1d

General procedure for synthesis of the new derivatives of cephalexin (1-4).

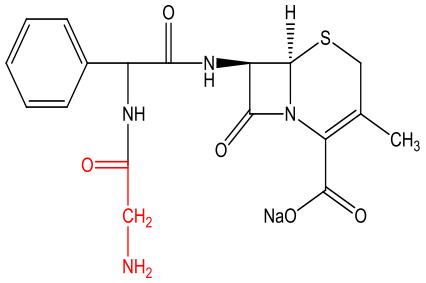
These compounds were obtained by deprotection [10] of the amino group of compounds **1a-d**, to afford the new derivatives of cephalexin 1-4, as follows and as shown in (schemes 3 and 4).

Compounds **1a-d**, (1.984 mmol) was suspended in dichloromethane (DCM) (10ml) and cooled

to 0 °C in an ice bath and TFA (15ml) was added with continuous stirring for 1hr at 0°C in presence of anisole (3ml). The completion of the reaction was monitored by TLC using the mobile phase methanol: chloroform (1:1). Diethyl ether (100ml) was added to the reaction mixture and the resulting precipitate was collected, suspended in methanol (30) ml and the pH was adjusted to 7 with 5% methanolic solution of NaOH. A precipitate was formed after the addition of ether, which was filtered and washed with acetone and recrystallized from ethyl acetate: petroleum ether (9:1). The precipitate was collected and dried in an oven at 50°C.

Synthesis of 1, 7-(2-(2-aminoacetamido)-2 phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-2-ene-carboxylate Sodium.

Compound **1** was obtained by deprotection of the amino group of compound **1a**, as follows and as shown in (scheme 3):

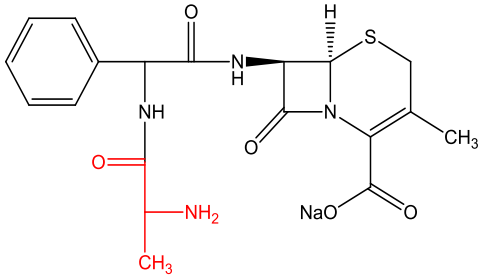


Chemical structure of compound 1

Compound **1a** (1.984 mmol, 1 g) in DCM (10ml) was reacted with TFA (15ml) in presence of anisole (3ml). The mixture was treated as previously described. A yellowish crystalline powder was collected. The physical appearance, percent yield and R_f value are listed on table (1).

Synthesis of 2, 7-(2-2-amino propan amido)2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo{4.2.0}oct-2-ene-carboxylate Sodium.

Compound **2** was obtained by deprotection of the amino group of compound **1b**, as follows and as shown in (scheme 3):

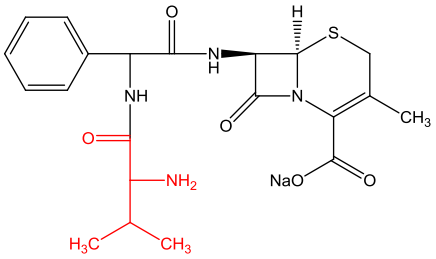


Chemical structure of compound 2

Compound **1b** (1.984mmol, 1.027 g) in DCM (10ml) was reacted with TFA (15ml) in presence of anisole (3ml). The mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed on table (1).

Synthesis of 3, 7- (2- (2-amino-3-methylbutan amido) 2-phenylacetamido)-3-methyl-8-oxo -5-thia-1-azabicyclo {4.2.0}oct-2-ene-carboxylate Sodium.

Compound **3** was obtained by deprotection of the amino group of compound **1c**, as follows and as shown in (scheme 3)**:**

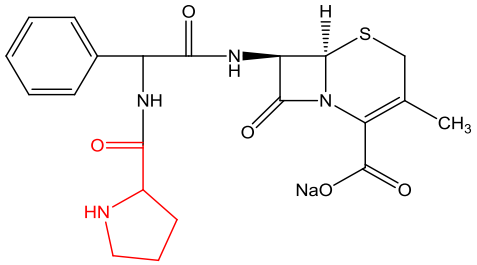


Chemical structure of compound 3

Compound **1c** (1.984 mmol, 1.083 g) in DCM (10ml) was reacted with TFA (15ml) in the presence of anisole (3ml) and the mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed on table (1).

Synthesis of 4, 7-(2-phenyl-2- (pyrrolidin -2 - carboxamido) acetamido)-5-thia-1-azabicyclo {4.2.0} oct - 2 - ene-carboxylate Sodium.

Compound **4** was obtained by deprotection of the amino group of compound 1d, as follows and as shown in (scheme 4):

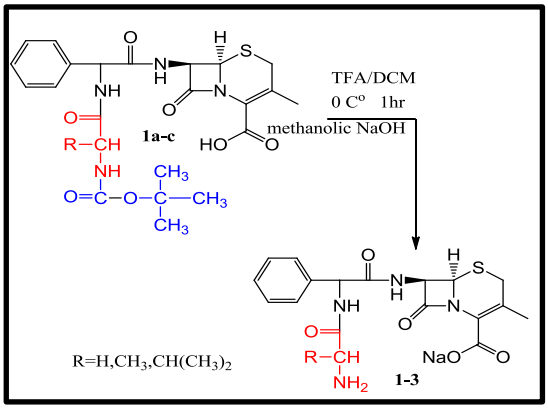


Chemical structure of compound 4

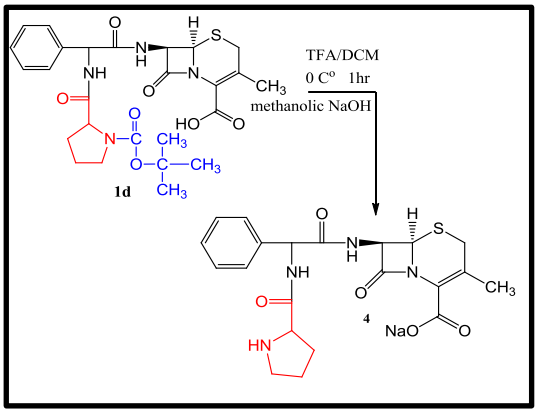
Compound **1d** (1.984 mmol, 1.081 g) in DCM (10ml) was reacted with TFA (15) in presence of anisole (3ml) and the mixture was treated as previously described. The physical appearance, percent yield and R_f value are listed in table (1).

Table (1): Physical parameters and percent yields of the synthesized compounds.

Compound	Physical appearance	% Yield	m.p. (^{o}C)	R_f value
1a	White	63.8	172-177	0.60 (A)
1b	White	72	182-186	0.44 (A)
1c	White	74	160-166	0.55 (A)
1d	Off white	82	180-185	0.50 (A)
1	Pale yellow	95	185 (decomposed)	0.41 (B)
2	Pale yellow	44	200 (decomposed)	0.46 (B)
3	Off white	70	214 (decomposed)	0.57(B)
4	Off white	40	212 (decomposed)	0.55(B)
Cephalexin	Off white	--	182-186	0.11(A)



Scheme (3): Synthesis of the target compounds 1-3



Scheme (4): Synthesis of the target compound 4

Results and Discussion

The use of two solvent systems A and B was to differentiate between reactants and products and to follow progress of reactions. A-Chloroform: Methanol (3:1) B-Chloroform: Methanol (1:3), as illustrated on Table (1).

The IR characteristic bands of compound **1** are 1543, 1400 (C=O stretching of carboxylate anion), 3060 (N-H stretching 2-amide, 3473, 3397 (N-H stretching of primary amine group), 1656 (C=O stretching 2-amide), 1761(C=O stretching β-lactam), 1543(N-H bending 2-amide). The elemental analysis (CHN) was calculated for $C_{18}H_{19}N_4SO_5Na$ (426); calculated: C; 50.70, H; 4.46, N; 13.145. Found C; 48.35, H; 4.684, N; 13.36.

The IR characteristic bands of compound **2** are 1549, 1400 (C=O stretching of carboxylate anion), 3061 (N-H stretching 2-amide), 3494, 3399 (N-H stretching primary amine group), 1664 (C=O stretching 2-amide), 1759 (C=O stretching β-lactam), 1527 (N-H bending 2-amide). CHN analysis was calculated for $C_{19}H_{21}N_4SO_5Na$ (440); calculated C: 51.8, H: 4.77, N: 12.72. Found C: 49.91, H: 4.56, N: 12.28.

The IR characteristic bands of compound **3** are as follows; 1543, 1400 C=O stretching of carboxylate anion, 3063 (N-H stretching of 2-amide), 3473, 3397 (N-H stretching of primary amine), 1653 (C=O stretching of 2-amide), 1759 (C=O stretching of β-lactam), 1570 (N-H bending of 2-amide). CHN analysis was calculated for $C_{21}H_{25}N_4SO_4Na$ (429) calculated; C: 53.846, H: 5.341, N: 11.965. Found C: 52.04, H: 5.16, N: 11.71.

The IR characteristic bands of compound **4** are 1550, 1400 C=O stretching of carboxylate anion, 3034.13N-H stretching of 2-amide, 3373, 3317 N-H stretching of primary amine group, 1662.69 C=O stretching of 2-amide, 1755.28 C=O stretching of β-lactam, 1527 N-H bending of 2-amide. CHN analysis was calculated for $C_{21}H_{25}N_4SO_5Na$ (445), calculated; C: 54.663, H: 3.904, N: 12.147. Found C: 52.20, H: 3.78, N: 12.68.

Preliminary Antimicrobial Evaluation

The synthesized compounds were subjected to antimicrobial evaluation by well-diffusion method [(12)]. The zone of inhibition (mm) was measured in comparison with cephalexin. The antimicrobial activity was performed in nutrient agar medium containing *E. coli, P. aeruginosa* and *Bacillus cereus, S. aureus* and the compounds used at concentrations (125 and 250 µg/well).The activity was determined after incubation for 24h at 37^{o}C by the comparison of inhibition of growth of bacteria by cephalexin using dimethyl sulfoxide (DMSO) as the solvent. No inhibition zone was observed for the control (dimethylsulfoxide). These compounds were subjected to preliminary antimicrobial evaluation against four types of microbes (*E. coli, P. aeruginosa*, and *Bacillus cereus, S. aureus).*

Table (2) :The preliminary antibacterial activity of the new derivatives of cephalexin.

Compound	Concentration µg/ml	Escherichia coli.	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus cereus.
DMSO	--	**9**	-	**10**	-
cephalexin	**125**	**10**	-	**12**	-
	250	**12**		**14**	
1	**125**	-	**11**	-	-
	250	-	**12**	-	-
2	**125**	-	**11**	-	**6**
	250	-	**14**	**8**	**10**
3	**125**	**8**	**15**	**9**	**15**
	250	**9**	**15**	**9**	**17**
4	**125**	-	-	-	-

Key to symbols: (-) = no inhibition.

The antimicrobial evaluation revealed that the newly synthesized compounds**, 1-4** showed reasonable antibacterial activities against G (-) bacteria, such as *P. aeruginosa and* G (+) bacteria, such as *Bacillus cereus* in comparison with cephalexin, which has no activity against these type of microbes.

Compound **1** (125 and 250µg) showed significant activity against *P. aeruginosa* and reduction in antibacterial activities against *S. aureus* and *E. coli* as compared with cephalexin. Compound **2** (125 µg, 250µg) showed significant activity against *P. aeruginosa* and *Bacillus cereus*, as compared with cephalexin. Furthermore, at concentration (125 µg) compound **2** exhibited no activity against *S. aureus* and *E. coli*. However, at concentration of (250 µg) the activity was less against *S. aureus* as compared with cephalexin. Compound **3** (125 and 250µg) showed very significant activity against *E. coli, P. aeruginosa* and *Bacillus cereus* and slight activity towards *S. aureus*. This result indicates that compound **3** has a broader spectrum of antibacterial activities, i.e. against both G (+) and G (-) bacteria.

Compound **4** (125µg) has no antibacterial activities against all strains of bacteria used. However, Compound **4** at concentration (250 µg) showed significant activity against *P. aeruginosa* and no antibacterial activities against *E. coli, S. aureus* and *Bacillus cereus* as compared with cephalexin. This chemical modification at the acyl side chain positioned at alpha to β- lactam ring supposed to provide some protection against β-lactamses. This model contains a primary amino group of the amino acid, which may provide certain stability in the acidic condition of stomach [(4).]

Conclusion

A series of new derivatives of cephalexin have been synthesized successfully in appreciable yields and screened for their antimicrobial activity using well diffusion method against bacterial strains (*E. coli, P. aeruginosa* and *Bacillus cereus, S. aureus).*

It is concluded that the new derivatives of cephalexin linked with certain amino acids were found to possess moderate antibacterial activities. Furthermore, the new derivative of cephalexin linked with valine has a significant activity against *p. aeruginosa* and *Bacillus cereus.*

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